Renata Kasprzyk

ABSTRACT OF DISSERTATION

Methods of studies on proteins interacting with 5' mRNA end based on nucleotide molecular probes

Messenger RNA (mRNA) is a macromolecule representing a copy of genetic information that undergoes translation in order to produce a protein. The 5’ mRNA end, so called cap, is an untypical nucleotide structure consisting of 7-methylguanosine linked by a 5’-5’ triphosphate bridge with the first transcribed nucleotide. Due to the presence of a positive charge within the 7-methylguanosine ring cap specifically interacts with proteins participating in essential biological processes, such as mRNA maturation, nucleocytoplasmic transport, and translation initiation, and promotes transcript survival. Cap binding proteins play important regulatory role in gene expression and they are often of therapeutic interest, making 5’ cap an interesting research target. Monitoring of processes involving cap is possible using e.g. fluorescent labelling. Fluorescently labelled cap analogs act as molecular probes, consisting of one part responsible for specific interactions (cap) and other generating measurable analytical signal (fluorescent tag).

The main aim of the project was to apply simple synthetic methods to obtain versatile tools to develop fast and efficient fluorescent assays for studies on such therapeutic targets as: eukaryotic initiation translation factor 4E (eIF4E), decapping scavenger enzyme (DcpS) and mRNA cap N7-guanine methyltransferase (N7-MTase). Based on fluorescence changes appearing upon cap binding, hydrolysis or synthesis it is possible to determine physical parameters representing nucleotide–protein interactions. Moreover, high–throughput screening (HTS) methods can be utilized for screening experiments of small-molecule ligand libraries with possible antitumor (eIF4E, N7-MTase) or antiviral (N7-MTase) properties, or that can be used in treatment of genetic diseases, such as spinal muscular atrophy (DcpS). The project is interdisciplinary and combines such fields of science as nucleotide synthesis, spectroscopy, biophysics and physics of carbon nanostructures.

In the first part of the dissertation a short description of biological role of 5’ cap is presented, including characteristics of proteins such as eIF4E, DcpS and N7-MTase, which were the main focus of the studies. The next chapter explains the basics of methods especially important for the project. Fluorescent techniques, such as fluorescence intensity, fluorescence polarization/anisotropy (FP/FA) and microscale thermophoresis (MST) were depicted as well as their potential applications. In similar manner methods useful for studies on conjugates of nanomaterials with biological molecules were characterized (infrared, Raman and X-ray photoelectron spectroscopies). In the next section the main results of the project were described. Four methods in total based on pyrene–nucleotide quenching interactions were developed and adapted to HTS format. Some synthesized probes were used to develop MST methods and FA assay for studies on protein involved in nucleotide metabolism (transglutaminase II). Then, the concept of double–labelled nucleotide analogs relying on interactions between two fluorescent tags was introduced. Three sets of nucleotides using excimer formation, FRET or fluorophore–quencher interactions were characterized spectroscopically and enzymatically to exhibit their

potential *in vivo* applications. The final part of results describes attempts of chemical modifications of graphene flakes exfoliated on silicon carbide and synthesis of cap–graphene oxide conjugate as parts of development of biosensor designed for eIF4E protein (tumor marker) concentration determination using changes of conductivity. Experimental section consists of detailed protocols of nucleotide synthesis, spectroscopic measurements and physical parameters calculations. The last chapter contains the list of literature cited throughout the dissertation.